Mapping of Gene Mutations in *Drosophila melanogaster*

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Abstract:

In this experiment, mutant genes of a given unknown mutant strain of *Drosophila melanogaster* were mapped to specific chromosomes. *Drosophila melanogaster,* commonly known as the fruit fly, was the appropriate choice for the organism to use in this specific experiment because of its relatively rapid life cycle of 10-14 days and because of the small amount of space and food neccessary for maintaining thousands of flies. The *D. Melanogaster* unknown strain specifically used in this experiment was arbitrarily named Amanita. This unknown was phenotypically characterized by dark body(head, thorax, and abdomen) as opposed to light body in the wild-type, white eyes as opposed to red eyes in the wild-type, and a wing with a shortened fourth longitudinal wing vein as opposed to a complete fourth longitudinal wing vein in the wild-type. All other traits were found to be the same as in wild-type *D.melanogaster*. Amanita was found to carry four mutant genes, two controlling eye color, one controlling body color and one controlling wing vein mutation.

Table 1: Summary of Gene Names

Gene name	Description	Gene Symbol
Dark Body	Darkened Body	dk
Bridal	White Eyes	wh
Autowhite	Brown Eyes	br
Short Vein	Shortened vein #4	vn

All of the noted mutant genes are recessive. The gene vn was found to be located on chromosome two. The genes dk and br were found to be genetically linked and located

on autosomal chromosome three. The gene wh was found to be sex-linked and located on the x-chromosome. Eye color involves epistasis, with white epistatic to brown. Brown is only seen when br is homozygous recessive and wh is wild-type(wh+).

Below are the maps of the three chromosomes which include both unknown mutant genes and known marker genes

X chromosome

---___wh_____12.1m.u._____cv__38.6m.u._____f-----
1.6 13.7 52.3

Chromosome 2

---_____vn_____35.2m.u._____Bl_____26.6m.u._____L---
19.6 54.8 81.4

Chromosome 3

__br_____11.8m.u.___Ly__19.4m.u._____Sb_14.9m.u._____dk____

28.7 40.5 59.9 74.8

Introduction:

Drosophila melanogaster, the common fruit fly, has been used for genetic experiments since Thomas Hunt Morgan's experiments in 1907. *Drosophila melanogaster* make good genetic specimens because they have simple food requirements, only have four pairs of chromosomes, they are small and easy to raise in the lab, they produce many offspring, have a short life-cycle(10-14 days), and have easily discernable mutations. Mutations are changes in the chromosomes, genes, or part there of resulting in a change in the characteristics of an organism. Mutations can be caused by large changes in chromosome arrangement such as inversions and deletions. Inversions are structural aberrations in a chromosome in which the order of several genes is reversed from the normal order, whereas deletions are losses of segments of the genetic material from a chromosome. There may also be smaller mutations where there is only a single base pair change in the DNA, this is called a point mutation. Mutations occur spontaneously in nature. Most mutations produce weakness that can be observed in the lab, such as abnormal body morphology, shortened life span, sterility, or death. In the natural world, most mutated flies would die out, whereas in the lab they can be maintained over many generations under lab conditions. Many mutant genes are displayed in the phenotype only when both genes in the pair are affected, meaning that the mutation would be recessive in this case. When only one gene is affected and mutations are displayed in the phenotype, the mutation would be dominant. Sex-linked(X-linked) mutations are carried on the X-chromosome, whereas autosomal mutations are carried on autosomal chromosomes. Autosomal chromosomes are all chromosomes other than the sex chromosomes.

The purpose of this particular exeriment is to determine the genetic constitution, the number and location of mutant genes, of an unknown mutant strain of *Drosophila melanogaster*. The unknown strain of *Drosophila melanogaster* was arbitrarily named amanita. This unknown was phenotypically characterized by dark body(head, thorax, and abdomen) as opposed to light body in the wild-type, white eyes as opposed to red eyes in the wild-type, and a wing with a shortened fourth longitudinal wing vein as opposed to a complete fourth longitudinal wing vein in the wild-type. All other traits were found to be the same as in wild-type *D.melanogaster.*

In order to determine the genetic constitution of amanita, a series of genetic crosses must be carried out. The first crosses, 1A and 1B, are designed to determine modes of inheritance of mutant genes and to determine whether or not genes are assorting independently. The modes of inheritance of the mutant genes, whether they are dominant or recessive, sex-linked or autosomal, can be determined upon examination of the phenotypes present in the F1(first generation of descent from a given mating). The F2(the second generation of descent, produced by intercrossing F1 organisms) 1A and 1B progeny must be examined in order to determine sex linkage v. autosomal inheritance, the number of genes involved, and whether or not genes are segregating independently. Independent segregation is the random distribution of unlinked genes into gametes. The unlinked genes can either be genes on different chromosomes or genes that are far apart on the same chromosome. Two genes that are on separate chromosomes assort independently and display a 9:3:3:1 dihybrid(ratio seen in the progeny of a cross between homozygous strains that differ at two genetic loci) ratio. Genes that are physically(greater than 50 map units apart), but not genetically linked(less than 50 map

units apart) display a 5:1:1:1 dihybrid ratio. A chi square test is used to test for independent assortment, physical linkage, and genetic linkage.

The next series of crosses, termed the male parent backcrosses(MPBC), must be conducted in order to determine on which chromosome each mutation is present. It is a given at the outset of the experiment that none of the mutations are located on chromosome 4. Since the 1A and 1 crosses determine which mutations are X-linked, it is unnecessary to analyze the X chromosome here. In the MPBCs, F1 males from a cross of parental amanita males and marker stock females (marker stock= flies with known locations of mutations) are crossed with amanita females. In *Drosophila melanogaster* crossing-over(recombination) only occurs in females, the reason for this is not known. Since males do not recombine and since the marker stock mutations are dominant mutations with known locations on specific chromosomes, the results of the MPBC will tell us which chromosomes the amanita mutations are on.

Bl L/Cy stands for short thin bristles, small eyes(lobed), and curly wings and these mutations are located on chromosome two(Bl locus 2-54.8, L locus 2-72.0, Cy locus 2 (associated with an inversion)). The gene which codes for curly is located on one of the pair of the homologous chromosomes and Bl/L is on the other. Any homozygous fly is non-viable because all three of the genes are dominant and homozygous lethal. The genes are pleiotropic, a condition in which a single mutant gene affects two or more distinct and seemingly unrelated traits, because they control both morphology and survival. Marker Stock 2 flies are all heterozygous because, of the three phenotypes that are produced during mating, two die(one which is homozygous for bristle and lobe and the other which is homozygous for curly) and the one that lives is the same as the

parental(heterozygous for all three traits). LySb stands for thin, cut wings(lyra) and short blunt bristles(stubble) and these mutations are located on chromosome three(Ly locus 3-40.5, Sb locus 58.2). LVM is another non-visible mutation and it is present as a complement to Ly and Sb. This is another example of pleiotropy. All three genes are dominant and lethal when homozygous. MS 3 is a heterozygote for the same reasons as MS2. LVM and Cy are called balancer chromosomes and they allow for successful use of dominant mutations in gene mapping. When balancers are present that homologous pair can not undergo recombination and therefore the parentals are maintained.

Maps of positions of linked genes on a chromosome can be constructed by calculating the frequencies of cross-over between genes(recombination frequency). During meiosis, each homologous pair of chromosomes becomes doubled, forming 2 sets of sister chromatids, termed a tetrad. During the first phase of meiosis, prophase 1, the strands of the tetrad overlap one another and twist around each other. Non-sister chromatids may exchange segments at this time and this is termed crossing-over. The closer two genes are together, the less likely is cross-over. The greater the distance between two genes on a chromosome, the greater the chance of cross-over. The probability of cross-over can be expressed as a distance or value, the% of crossovers that occur between two points on the chromosome. One map unit is the distance between linked genes in the space where 1% of crossing-over occurs.

The last series of crosses, the female parent back crosses (FPBC) must be carried out in order to determine the specific location of the mutant genes to the chromosomes that they were determined to be on in the MPBC. During the FPBC, amanita males are mated to MS females of the F1 generation in order to determine recombination

frequencies, which will be used to map the unknown mutant genes. Unlike the MPBC, notable recombination does occur in the FPBC and this allows us to be able to map the genes.

Materials and Methods:

The materials required to conduct this experiment were culture(males and females with the same true-breeding genotypes) bottles of wild-type *Drosophila melanogaster*, culture bottles of three known mutant *Drosophila* melanogaster strains and culture bottles of an unknown mutant strain of *Drosophila melanogaster*. The following materials were also needed: bottles with food at the bottom, vials, counting plates, ether bottles, etherizers, funnels,re-etherizers, sponge pads, dissecting picks, a dissecting microscope and a fly morgue.

The first step of the experiment was to examine wild-type *Drosophila melanogaster*. In order to look at and manipulate the flies it was necessary to etherize them. A few drops of ether were placed on the cotton at the bottom of the etherizer jar, the ether fumes were allowed to dissolve into the jar for a few minutes and then a funnel was placed into the jar. The wild-type flies were tapped into the bottom of their culture bottle, the cap was removed off of the culture bottle and the culture bottle was quickly inverted into the funnel. After the flies stopped moving around, they were poured onto the counting plate and examined under the dissecting microscope. The following main adult body parts and segments were observed:

Head:

1. Antennae-consisting of three segments.

2. A branched, two-jointed arista, arising near the base of the distal segment of each antenna.

3. Proboscis.

4. Compound eyes composed of a large number of ommatidia.

5. Ocelli-simple eyes, three in number, situated between the compound eyes on the dorsal aspect of the head.
6. Bristles-orbital and post-vertical.

Thorax:

1. Prothorax-with the first pair of legs attached.
2. Mesothorax-consisting of the dorsally situated mesonotum and scutellum, the wings, and the second pair of legs.
3. Metathorax-containing the halteres and the third pair of legs.
4. Bristles-dorsocentral and scutellar.
5. Legs-consisting of coxa, trochanter, femur, tibia, five tarsal joints and sex comb on proximal tarsal joint of the male.
6. Wings-complete venation.
7. Halteres(balancers)-highly modified wings, each with three segments.

Abdomen: There were seven or eight visible segments in the female and five in the male:

1. Tergites.
2. Sternites-six in the female, four in the male.
3. Female genital region, with anal plates and ovipositor plates.

4. Male genital region-anal plates, genital arch, claspers, penis.

The flies were then sexed. The females were distinguished by the elongated tip of the abdomen, the pattern of lighter markings on the abdominal segments, and the seven abdominal segments. The males were distinguished by the rounded abdomen, the pattern of darker markings on the abdominal segments, the five abdominal segments and the sex-combs(a fringe of ten stout lack bristles on the distal surface of the basal tarsal joint of the first pair of legs).

The flies that awoke during examination were re-etherized by putting ether on the gauze of the re-etherizer and by placing the re-etherizer over them. After they were done being examined, the flies were placed into the fly morgues.

The next step of the experiment was to examine the unknown strain of *Drosophila melanogaster*, arbitrarily named amanita, and to compare and contrast amanita to the wild-type. The unknown strain was examined by the same etherization method described previously. Amanita was noted to differ from the wild-type in the following aspects: amanita had white eyes as opposed to red in the wild-type, dark body color as opposed to light in the wild type, and a missing distal fourth longitudinal wing vein as opposed to complete venation in the wild-type. These flies were discarded into the fly morgue after examination.

The next step of the experiment was to set up various crosses(males and virgin females of different genotypes) for the dihybrid analysis. The first crosses of the dihybrid analysis were the following:

1A	and	1B
P1 15 amanita female X	15 wild-type male	P1 15 wild-type female X 15amanita male

(P1=parental)

The crosses were set up in duplicates, so there were two bottles of the 1A cross and two bottles of the 1B cross. In order to make these crosses, flies from both the amanita culture bottles and the wild-type culture bottles were etherized and sexed. Thirty virgin amanita females and thirty amanita males were isolated from the amanita culture bottles while thirty wild-type males and thirty virgin wild-type females were isolated from the wild-type culture bottles. The etherized flies were then placed in their respective cross bottles. When making each new cross, the cross bottle was placed on its side and the etherized flies were carefully placed on the side of the bottle. Each bottle was capped and left on its side until the flies recovered from the anesthetic so as to avoid the etherized flies getting stuck in the food at the bottom of the bottle. It was very important that the females collected were virgins. Since females do not copulate for ten-twelve hours after emerging from the puparium, the safest way to make sure that the females being collected are virgins was to clear the culture bottles of all flies that eclosed, place these in the fly morgue and to return to the lab to collect the virgin females that had eclosed within eight hours of clearing the culture bottles.

When pupae became evident, the parents of the 1A and 1B crosses were cleared(6 days after the cross was set up) into the fly morgue. After a few days, the F1(first generation of descent from a given mating) generation emerged from the puparia and 200 flies were scored(etherized, examined, and the phenotypes of each sex from each cross were noted)from the 1A cross and 200 were scored from the 1B cross. After being scored, the flies were placed in the fly morgue.

The next crosses of the dihybrid analysis were:

15 1AF1 females X 15 1A F1 males and 15 1BF1 females X 15 1BF1 males These crosses were also set in duplicates, so there were two bottles of each. It was not neccessary that the females be virgins in these crosses. The procedure, described above, of obtaining the flies and placing them in their respective cross bottles, subsequently clearing the parents and scoring the F2(the second generation of descent, produced by intercrossing F1 organisms) generation was followed. 750 flies were scored from the 1AF2 and 900 from the 1BF2 and were then dumped in the fly morgue.

The next step of the experiment was examine three marker stocks(MS), marker stocks 2, 3, and 4. Marker or known stocks have known locations of gene mutations. Marker stock 2 has the following mutations(known to be on chromosome 2): short bristles(BL)(2/3 normal length)at locus 2-54.8, curled wings(Cy)at locus2-(associated with an inversion) and reduced eye size(L) at locus2-72.0. Marker stock 3 has the following mutations(known to be on chromosome 3): narrowed wing(Ly)at locus 3-40.5, bristles <1/2 length(Sb)at locus 3-58.2, and LVM at locus 3(associated with an inversion) and marker stock 4 the following mutations(known to be on the X chromosome): crossveins on wings absent(cv) at locus X-13.7 and shortened, bent bristles(f)at locus X-56.7.

The next step of the experiment was to set up various crosses between each of the marker stocks and amanita as the first step of the crossing scheme for gene mapping. The first crosses of this scheme were the following:

P1 15 MS2 females X 15 amanita males

P1 15 MS3 females X 15 amanita males

P1 15 MS4 females X 15 amanita males

These crosses were also set up in duplicate, so there were two bottles of each cross. It was important that all of the P1 females were virgins and to make sure of this the females of each marker stock were stored in separate vials(there were 5 females in each vial) from the males(15 males were stored in each vial) for three days until it was certain that there were no larvae present in any of the vials. There were no larvae present, so after three days the crosses were made. Except for the isolation of females for three days prior to starting the cross, the methods of this cross were the same as the previously described crosses(the flies were obtained and placed in their respective bottles, subsequently cleared and the F1 generation was scored). 50 F1 flies were scored from each cross and then dumped in the fly morgue.

The next crosses of the crossing scheme for gene mapping were the male and female parent backcrosses:

Cross	Female Parent		Male Parent
MS2 backcross, male parent	15amanita	X	15 F1 BL L, not Cy
MS2 backcross, female parent	15 F1 BL L, not Cy	X	15 amanita
MS3 backcross, male parent	15amanita	X	15 F1 Ly Sb
MS3 backcross, female parent	15 F1 Ly Sb	X	15 amanita
MS4	15 F1	X	15 F1

From the MS2 F1, only the BL L flies were used for the male and female backcrosses, while the Cy flies were discarded. Likewise, from the MS3 F1 generation, only the LySb flies were kept while the wild-type flies were discarded. The first four crosses were also set up in duplicates, so there were two bottles of each cross. Four cross bottles were

set up for the MS4 F1 X F1. The same methods were followed for setting up these crosses as described previously for the other crosses. 96 F2 progeny were scored for the MS2 male parent backcross, 67 F2 progeny were scored for the MS3 male parent backcross, 508 F2 progeny were scored for the MS2 female parent backcross, 744 for the MS3 female parent backcross, and 586 for the MS4 F1 cross. The flies were then discarded in the fly morgue.

All culture and cross bottles form the lab were discarded at this point.

Results:

I. Crosses 1A and 1B

Table1: Phenotypes of the F1 generation of 1A and 1B crosses

	1A	1A	1B	1B
F1	Females	Males	Females	Males
Eye color	+	White	+	+
Body color	+	+	+	+
Wing veins	+	+	+	+

+=wild-type

From the 1A cross of amanita virgin females to wild-type males, only red-eyed F1 females and white-eyed F1 males are seen. From the 1B cross of wild-type virgin females to amanita males, only red-eyed F1 females and males are seen. These results prove that the eye color gene is sex-linked and recessive, since only white-eyed males show the white phenotype. The wh allele, which is homozygous in the amanita female(whwh), can combine with either the wh+allele on the wild-type male's X-chromosome or with the male's Y chromosome which does not carry either allele of the wh gene, giving the progeny of heterozygous wild-type females(wh+wh) and white-eyed males(wh_). The fact that the F1 1A females have red eyes and the fact that the F1 males(wh+_) and females(wh+wh) of the 1B cross all have red eyes shows that the wh allele is recessive.

For both reciprocal crosses, the F1 male and female progeny have light body color and a complete fourth longitudinal wing vein. Since there is no difference between the

sexes in either reciprocal cross, this proves that both the gene for body color and the gene for wing vein are located on one of the autosomal chromosomes(at this point it is not possible to tell if the genes are located on the same or on different chromosomes). Since the light body color is seen over the dark body color in all F1 progeny, the dark body color allele(dk) is recessive. Since the complete fourth longitudinal wing vein is seen over the shortened fourth longitudinal wing vein, the shortened wing vein allele(vn) is recessive.

Table 2: Phenotypes of the F2 generation of 1A and 1B crosses

	1A	1A	1B	1B
F2	Females	Males	Females	Males
Eye color	Red, brown, white	Red,brown, white	Red, brown	Red, brown, white
Body color	Light, dark	Light, dark	Light, dark	Light, dark
Wing vein	Wild-type, mut.	Wild-type, mut	Wild-type, mut	Wild-type, mut

Mut=fourth longitudinal wing vein shortened

The equation 2*n=x, where x is the number of phenotypes and n is the number of genes, is used to analyze the number of genes controlling the specific phenotype. Since there are two phenotypes for both body color and wing vein, there is only one gene controlling each trait. There is no distinction between males and females in the F2 generation for body color and wing vein and therefore it is confirmed that the dk and vn alleles are autosomal. There are three phenotypes for the eye color, so 2*n=3, and therefore there are 2 genes controlling eye color. But there are only three phenotypes observed and not four. This can be explained by epistasis, where one eye color gene is

masking the other phenotype. Epistasis is a situation in which the genotype at one locus determines the phenotype in such a way as to mask the genotype present at a second locus. It can not be a pleiotropic gene since the homozygous amanita parents are viable. None of the 1B F2 females have white eyes, but all other F2 progeny do. This shows that the white-eye allele(wh) must be sex-linked. Brown eyes are inherited for all F2 progeny of both the 1A and 1B crosses, so the brown-eyed allele(br) must be autosomal. Therefore, there are two genes controlling eye color, one that is X-linked(wh) and causes white eyes when homozygous and one that is autosomal(br) and causes brown eyes when homozygous. The X-linked white-eyed gene is epistatic to the autosomal brown eyed gene.

From this data, it can also be determined whether two genes are on the same or different chromosomes. Genes that are on separate chromosomes will assort independently and display a 9:3:3:1 dihybrid ratio. Genes that are physically linked (greater than 50 map units apart), but not genetically linked will show a 5:1:1:1 ratio.

These ratios must be corrected for fitness of the mutant alleles. Mutant alleles, for the most part, reduce the *Drosophila melanogaster's* ability to survive. Therefore, mutant flies are less likely to survive to be scored and would not realize their potential ratios. In order to test for these 9:3:3:1 and 5:1:1:1 ratio's, we must correct them for the fitness of the mutant genes. This is done by applying observed monohybrid ratios of each gene pair to the 9:3:3:1 and 5:1:1:1 expected ratios to produce new expected ratios.

Below are the observed monohybrid ratios for the mutant alleles when compared to wild type. 1BF2 females are excluded when calculating ratios involving wh. Since

white is epistatic to br, it can not be determined if br is mutant or wild-type when with homozygous recessive white, so these progeny are excluded in ratios involving br.

Table 3: Observed Monohybrid Ratios

Gene Name	Genotype Ratio (wild/mutant)	Reduced Ratios (wild/mutant)
Dark Body	dk+_ / dkdk	3.92 to 1
Short vein	vn+_ / vnvn	3.16 to 1
Autowhite	br+_ / brbr	3.98 to 1
Bridal	wh+_ / whwh	1.26 to 1

From these monohybrid ratios, the expected dihybrid ratios can be determined in the table below. Since the white eyed flies can not be determined to be brown or red-eyed at the autosomal eye color gene, they are combined into one category.

Table 4: Expected Dihybrid Ratios

Gene Combo			Ratios								
Dark Body (dk)	to	Short Vein (vn)	12 dk+_ vn+_	to	3.1 dkdkvn+_	to	3.9 dk+_ vnvn	to	1 dkdk vnvn		
Dark Body (dk)	to	Autowhite (br)	16 dk+_ br+_	to	3.9 dkdkbr+_	to	3.9 dk+_brbr	to	1 dkdk brbr		
Dark Body (dk)	to	Bridal (wh)	4.9 dk+_ wh+_	to	1.2 dkdkwh+_	to	3.9 dk+_ whwh	to	1 dkdk whwh		
Short Vein (vn)	to	Autowhite (br)	13 vn+_ br+_	to	3.9 vnvnbr+_	to	3.1 vn+_ brbr	to	1 vnvn brbr		
Short Vein (vn)	to	Bridal (wh)	4 vn+_ wh+_	to	1.26 vnvnwh+_	to	3.1 vn+_ whwh	to	1 vnvn whwh		
Autowhite (br)	to	Bridal (wh)	5 br+_ wh+_	to	1.26 brbrwh+_	to	4.9 __ whwh	cannot	distinguish		

These expected dihybrid ratios are then compared to the observed dihybrid ratios using a chi square test. If the comparison fails such a test, we reject the null hypothesis, and the genes are either genetically or physically linked. If, however, the genes pass the chi square test, then we must accept the null hypothesis to show that the genes are assorting independently. If two genes pass the chi square test, they are assorting independently and are therefore on separate chromosomes. The table below summarizes the results of the chi squared test for independence.

Table 5: Chi Square Results

Sum X^2 (chi squared) (Df=3, p=.05, X^2 (cv)=7.82) *	**Gene Combination**			
0.493	Dark Body (d)	to	Short Vein (s)	Assort Independently (accept null hypothesis)
260.734	Dark Body (d)	to	Autowhite (b)	Linked (reject null hypothesis, genetic or physical linkage)
5.207	Dark Body (d)	to	Sexywhite (w)	Assort Independently (accept null hypothesis)
1.932	Short Vein (s)	to	Autowhite (b)	Assort Independently (accept null hypothesis)
1.168	Short Vein (s)	to	Sexywhite (w)	Assort Independently (accept null hypothesis)
1.005	Autowhite (b)	to	Sexywhite (w)	Assort Independently (accept null hypothesis)

*The calculations had 3 degrees of freedom since there were four classes. The value of 7.82 is the number in the p=0.05 column(the division line for accepting and rejecting hypotheses) for three degrees of freedom. The null hypothesis is rejected for sums>7.82, and accepted for values<7.82.

Only the dark body gene(dk) and the autosomal brown-eyed gene(br) are linked and on the same chromsome. The other genes are on separate chromosomes. It must then be determined whether the dk and br genes are genetically linked(less tham 50 map units apart) or physically linked(greater than 50 map units apart). The next null hypothesis is that the genes are physically linked, but genetically unlinked. In non-fly organisms, if genes are located more than 50 map units apart (physically but not

genetically linked) they will assort independently(9:3:3:1 ratio of progeny). Since male flies do not recombine due to reasons that are not known, they only donate parental chromosomes and the two genes should fit the 5:1:1:1 ratio of progeny for physical linkage, but not genetic linkage. The ratio is 5:1:1:1 because of the four female phenotypes formed upon recombination and the two possible male phenotypes. Therefore, there are eight possible phenotypes in the progeny that are reduced into the following four classes: 5dk+br+,1dkbr+,1dk+br, and 1 dkbr. The chi square test is again used to determine if the null hypothesis can be accepted, but the ratios are first fitness corrected to fit the 5:1:1:1 ratio. The sum of 51.77 is greater than the cv of 7.82, so the null hypothesis is rejected again, and the genes must not be physically linked. The two genes, dk and br, must therefore be genetically linked. Their recombination frequencies will be determined in the FPBC section.

In conclusion, the 1A and 1B crosses identified a total of four mutant genes: dk, vn, br and wh. The wh gene is determined to be X-linked and recessive. The recessive wh is epistatic to br and this causes the brown phenotype to be masked. The other three mutant genes are recessive and autosomal. The two genes dk and br are on the same chromosome and they are genetically linked, while vn is on a separate autosomal chromosome.

Male Parent Backcross:

From the MS2 and MS3 MPBC, the two following tables were obtained. Since it is already known that the epistatic wh gene is located on the X chromosome(X-linked), it is not necessary to examine the wh gene in the MPBC data.

Table 6: Male Parent Back Cross Data for Chromosome 2(MS2, Bl L)

unknown(amanita) virgin females X F1 Bristle males

Phenotype Observed	**Observed Number of Progeny**
Bl L br+ dk+ vn+	16
Bl L br dk vn+	28
Bl+ L+ br+ dk+ vn	21
Bl +L+ br dk vn	31

Table 7: Male Parent Back Cross Data for Chromosome 3 (MS3, LySb)

unknown(amanita) virgin females X F1 Lyra Stubble males

Phenotype Observed	**Observed Number of Progeny**
Ly Sb br+ dk+ vn+	25
Ly Sb br+ dk+ vn	10
Ly+ Sb+ br dk vn+	18
Ly+ Sb+ br dk vn	14

Each unknown mutation can then be analyzed separately. If there are two phenotypes for the marker stock gene and one unknown, then the two genes are linked to the same chromosome; there is not independent assortment occurring. However, if four phenotypes are observed, then the unknown gene is assorting independently of the MS genes and is therefore on a different chromosome.

In table 6, when dk is analyzed separately from the mutant wing (vn) and the autosomal eye color gene (br), there are 4 phenotypes. Hence we see that dk is assorting independently of Bl and L and is therefore not on chromosome 2. It can be deduced that dk must then be on chromosome 3 since it is already proven that it is not X-linked and

therefore not on the X-chromosome, not on chromosome 2 as stated above, and not on chromosome 4 (stated in the experimental outline). In table 7, when dk is analyzed separately, there are two phenotypes present. This indicates that dk is linked to Ly and Sb, and is therefore on chromosome 3 as was expected.

In table 6, when the autosomal eye color gene (br) is analyzed separately from the mutant wing (vn) and the body color gene(dk), there are 4 phenotypes. Hence, br is also assorting independently of Bl and L and is therefore not on chromosome 2. We can indirectly deduce, and confirm later that it must then be on chromosome 3 since it is already proven to not be on the X chromosome and to not be on chromosome 2. Again, it was a given in this experiment that none of the mutated genes were located on chromosome 4. In table 7, when br is analyzed alone, there are two phenotypes present. This indicates that br is linked to Ly and Sb, and is therefore on chromosome 3 as was expected. Note again that any white phenotype will mask any brown genotype due to epistasis. Therefore, when examining the brown (br) autosomal gene in MPBC data, all white-eyed flies were excluded to account for epistasis.

In table 6, the mutant wing gene (vn) shows only two phenotypes when analyzed with Bl and L. This shows that vn is linked to Bl and L and is therefore on chromosome 2. Table 7 shows 4 phenotypes when analyzed for vn, Ly and Sb. Therefore, vn is not on chromosome 3, since it is shown to be assorting independently. Moreover, vn was shown to be autosomal, so it cannot be on chromosome 1 (X). Moreover, it is given that no gene can be on chromosome 4 in this experiment. This information confirms that the mutant wing (vn) gene must be on chromosome 2.

These results can also be confirmed from the analysis of the 1A and 1B crosses. We found in both parts that vn and wh are assorting independently of all other genes and that dk and br are on the same chromosome (genetically linked).

Table 8: Summary of Genes Located on Specific Chromosomes

Gene	Chromosome
Dark Body(dk)	3
Autowhite (br)	3
Short Vein (vn)	2
Bridal (wh)	1 (x chromosome)

Female Parent Backcross:

Marker Stock 2:

For chromosome 2, there is only one unknown mutant gene(vn) present, so only one three point cross is needed to map the three genes (Bl, L and vn). Genes and their complements are added to determine which sets are parents, single recombinants and double recombinants. The results for vn are seen below.

Table 8: Marker Stock 2 Analysis

Chromosome 2	**(one three point cross needed)**	
Phenotype	**Number of Progeny**	**Category**
Bl L +	106	Parental
+ + vn	110	Parental
Bl + vn	10	DCO
+ L +	12	DCO
Bl L vn	80	SCO(I)
+ + +	77	SCO(I)
Bl + +	48	SCO(II)
+ L vn	65	SCO(II)
Total flies	508	

DCO=double crossover
SCO(I)= single crossover region I
SCO(II)=single crossover region II

Recombinants resulting from double crossovers are the least frequent events and the pair of chromosomes that yields the smallest number of progeny is chosen as the DCO class. Bl is the double crossover gene since this class yields the lowest number, so Bl must be in the middle of L and vn. This is the only way to recombine the parental class in the order of the double recombinant class by having two crossovers. The order must be vn-Bl- L, since it is known that L is located after bristle on chromosome 2. The recombination frequency between the genes in region 1 (vn to Bl) is calculated by adding the number of recombinants in that region(SCOs in region 1 +DCOs) and by dividing this number by the total number of flies. The recombination frequency for region II is calculated by adding SCOs in region II +DCO's and by dividing this number by the total number of flies. Recombination frequency is multiplied by 100 to get map units (m.u.) since 1% recombination equals one map unit. Once the map units are determined, they are assigned to a locus on the chromosome after a fixed marker is set. Bl is set to the known value of 54.8 and the other genes are mapped from that point.

Table 9: Marker Stock 2 Recombination Frequency.

Recombination Frequency			Map units
Bl and L	(10+12+48+65)/508	=	**26.60%**
vn and Bl	(10+12+77+80)/508	=	**35.20%**

Chromosome 2:

vn 35.2 m.u. Bl 26.6 m.u. L

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19.6 54.8 81.4

The coefficient of coincidence (COC) is determined to be .46 and the interference is .54. The COC is calculated by dividing the frequency of DCO's by the expected # of DCOs. Interference is 1- COC. The observed number of crossovers are therefore less than expected because of chromosome interference, in which crossing over in one region reduces the probability of a second crossover in a region that is close to the first crossover region.

Marker Stock 3:

There are two unknown mutant genes on chromosome 3, dk and br. For marker stock 3 (chromosome 3), a minimum of two three point crosses are needed to match the 4 genes Ly, Sb, br, and dk(each unknown mutant gene is looked at separately). The unknown dk is first analyzed separately from br.

Table 10: Marker Stock 3 Analysis: Phenotypes used to map dk gene

Chromosome 3	**3 pt. Cross #1**	genes: Ly, Sb and dk
Phenotype	**Number of Progeny**	**Category**
Ly Sb +	245	Parental
+ + dk	258	Parental
+ + +	57	SCO(II)
Ly Sb dk	40	SCO(II)
Ly + dk	50	SCO(I)
+ Sb +	80	SCO(I)
Ly + +	8	DCO
+ Sb dk	6	DCO
total flies	744	

SCO(I)=single crossover region I

SCO(II)=single crossover region II

DCO=double crossover

As above, these are converted into recombination frequencies. Noting the DCO, Sb is found to be in the center of the other two genes. Hence the gene order must be Ly- Sb-dk, since it is known that Sb is located after Ly on chromosome 3.

Table 11: Marker Stock 3 Recombination Frequency Set 1

Recombination Frequency			Map units
Ly and Sb	(8+6+80+50)/744	=	**19.40%**
Sb and dk	(8+6+57+40)/744	=	**14.90%**

The unknown br is then analyzed separately from dk.

Table12: Marker Stock 3 Analysis: Phenotypes used to map br gene

Chromosome 3	**3 pt. Cross #2**	genes: Ly, Sb and br
Phenotype	**Number of Progeny**	**Category**
Ly Sb +	300	Parental
+ + br	222	Parental
Ly Sb br	39	SCO(I)
+ + +	40	SCO(I)
Ly + +	63	SCO(II)
+ Sb br	70	SCO(II)
+ Sb +	6	DCO
Ly + br	4	DCO
total flies	744	

DCO=double crossover

SCO(I)=single crossover region I

SCO(II)=single crossover region II

As above, these are converted into recombination frequencies. Noting the DCO, Ly is found to be in the center of the other two genes. Hence the gene must be br-Ly-Sb since it is known that Sb is located after Ly on chromosome 3.

Table13: Marker Stock 3 Recombination Frequency Set 2

Recombination Frequency			Map units
Ly and Sb	(4+6+63+70)/744	=	**19.20%**
br and Ly	(4+6+39+40)/744	=	**11.96%**

When we combine the two three point crosses, we get a map of the entire chromosome 3. Ly is fixed to 40.5 for reference.

Chromosome 3

br 11.8 m.u. Ly 19.4 m.u. Sb 14.9 m.u. dk

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28.7 40.5 59.9 74.8

The recombination frequency between br and dk is estimated to be 46.1 which agrees with the data from the 1A and 1B crosses that determined that the two genes were genetically linked (less than 50 map units apart). The COC for Sb is .65 and I=.35. The COC for Ly is .53 and I=.47. Interference is then greater for Ly. The COC for Sb of .65 means that the observed number of DCO was 65% of those expected if cross over events in the two regions were independent, 53% of that expected for independent crossovers was seen for Ly. Crossover of Ly and Sb affect each other.

Marker Stock 4:

The X chromosome contains the wh mutant unknown gene. F2 progeny of a cross between the F1 progeny of the original MS4 were identified and only the males were scored because they only have one x chromosome and this makes it possible to see the recessive traits. Only males are examined to determine the map units and recombination frequencies(see below).

Table14: Marker Stock 4 Analysis

Chromosome 1 (X chromosome) **(one 3pt. cross needed)**

Phenotype	Numer of progeny	Category
Cv f +	133	Parental
+ + wh	169	Parental

+ + +	34	SCO(II)
cv f wh	24	SCO(II)
cv + +	112	SCO(I)
+ f wh	101	SCO(I)
cv + wh	8	DCO
+ f +	5	DCO
total flies	586	

The smallest numbered recombinant class determines the DCO's and therefore cv must be the double crossover gene. The same method is used to determine the order of the genes, the recombination frequency and the interference as in chromosome 2. From the data, the order is determined to be wh-cv-f since it is known that f is located after cv on the X chromosome. The location of the cv gene is fixed to 13.7.

Table 15: Marker Stock 4 Recombination Frequencies

Recombination Frequency			Map units
Cv and f	(8+5+101+112)/586	=	**38.60%**
Sb and d	(8+5+24+34)/586	=	**12.10%**

X chromosome(chromosome 1):

wh 12.1 cv 38.6 f

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1.6 13.7 52.3

The COC is 0.47 and I= 0.53. Again, it is apparent that crossover in one region affects the probability of a crossover in another region on the same chromosome.

Discussion:

The focus of this experiment was to highlight how linkage analysis could be used to determine allelic relationships. It is important to know the map position of a gene relative to other loci. With this information , genes can be manipulated to create combinations of

genes that will reveal gene relationships. The main objective of the experiment was to determine the location of various mutations through their linkage relationship to each other and to other known marker mutations.

In conclusion, unknown mutant genes can be mapped to chromosomes with known marker genes. Amanita was found to carry four mutant genes, two controlling eye color, one controlling body color and one controlling wing vein mutation. All of the noted mutant genes are recessive. The gene vn was found to be located on chromosome two. The genes dk and br were found to be genetically linked and located on autosomal chromosome three. The gene wh was found to be sex-linked and located on the x-chromosome. Eye color involves epistasis, with white epistatic to brown. Brown is only seen when br is homozygous recessive and wh is wild-type(wh+). Epistasis means "standing over", the presence of one mutation stands over, or conceals, the effects of a different mutation. In flies with a wh mutation, it is impossible to determine if br is mutant or wild-type. The converse is not true.

There are many sources that can contribute to errors in this experiment. Female flies could have been mistaken for males, females might not have been virgins. It was difficult to score some of the mutant phenotypes and this could lead to incorrect calculations and analysis of data. This could explain the deviations of the known marker stock mutant genes from their known loci. In addition anesthetized flies could have gotten stuck if in the food if the vials were handled improperly.

In order to perform accurate linkage analysis, it is necessary to be familiar with Mendelian inheritance techniques, phenotype identification, anesthitization, chi-square analysis, and other genetic concepts in addition to proper lab technique. The ability to

score various phenotypes is crucial, without this skill it is possible to misidentify the unknown mutation. Incorrectly scored phenotypes can lead to incorrect acceptance or rejection of various null hypotheses. Mendelian ratios must be known because chi-square analysis depends on correct ratios for the various crosses.

Linkage is a key genetic concept that has important roles in metabolic pathways and morphology, not only in *Drosophila melanogaster*, but also in *Homo sapiens.*

Reference List:

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2. Demerec D, Kaufmann BP 1957 Drosophila Guide, 1st ed.,

Lab handouts and lab notes were used in the writing of this paper.